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I, ANNA MAIJA EVERETT, ACTING TEAM LEADER EXAMINATION SUPPORT & SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 0810 for a patent by THE UNIVERSITY OF NEWCASTLE RESEACH ASSOCIATES (TUNRA) LIMITED filed on 07 June 1999.

WITNESS my hand this
Twentieth day of June 2000

A M Everett

ANNA MAIJA EVERETT
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AUSTRALIA

PATENTS ACT 1990

PROVISIONAL SPECIFICATION

FOR THE INVENTION ENTITLED:-

"A METHOD OF DETERMINING POTENTIAL SUSCEPTIBILITY TO DEVELOPMENT OF ALTE AND/OR SIDS"

The invention is described in the following statement:-

TECHNICAL FIELD

The present invention relates to methods for determining predisposition to acute life threatening episodes (ALTE) and/or sudden infant death syndrome (SIDS) and in particular to methods of determining potential susceptibility to development of ALTE and/or SIDS by monitoring levels of IgA.

BACKGROUND

A great deal has been done to minimize the risk of SIDS by non-specific methods related to infant care. However, prevention using specific assays related to causal mechanisms has not been explored. Identifying a causal mechanism may be expected to make a major impact on SIDS outcome through general awareness, and if used in conjunction with non-specific nursing care. The development of new techniques for identifying infants at risk of SIDS could be a significant outcome.

Interest in this approach to the prevention of SIDS arose as a result of an unusual opportunity of observing a 'prospective' case of SIDS during a study of 250 normal infants [1]. The infants were followed from birth, measuring parameters of immune status in saliva. The key observation in the one child who died from SIDS was an extraordinarily high IgM level in conjunction with less highly elevated IgA levels in saliva, appearing after a mild respiratory tract infection, several weeks before the child suddenly died. This observation in a single case was consistent with post mortem studies showing large numbers of IgM containing plasma cells regularly and specifically in the trachea and gut of subjects dying from SIDS [2-5]. The level of IgM was much in excess of any small increases seen in matched infection control studies [6]. These observations raised the possibility that assay of IgM in saliva of infants with an upper

respiratory tract infection may be a very useful marker of risk of SIDS, reflecting the disturbed mucosal immunoregulation that underpins the risk.

The numerous epidemiological studies of SIDS have identified many of the risk factors of SIDS but have failed to find a cause [7]. The role of infection and disturbed immunity has been proposed as one of the potential mechanisms for SIDS [8]. The common findings at autopsy of SIDS infants are consistent with infection or inflammation as a contributing cause of death [9]. SIDS has been reported to occur after a mild upper respiratory tract infection (URTI) [9-12], however there is no evidence that favours infections by an virulent pathogen. A low grade pathogen, that results in overstimulation of the immune system may be one important link in the chain of events that culminates in respiratory arrest.

There is evidence from post mortem studies [2-5, 13-14] and a prospective case study [1] of a gross disturbance of mucosal immunity in SIDS associated with prior respiratory illness or inflammation. These studies suggest an infective agent is responsible for the disturbance observed in the immune parameters.

Infants presenting with episodes of apnoea from which the infant recovers are termed acute life threatening episodes (ALTEs) and are classified as "near-miss" SIDS. It is clear that ALTE children could be expected to have a similar pattern of dysregulation of mucosal immunity to SIDS children.

To date, however, no method exists by which a prediction of the potential susceptibility to development of ALTE and/or SIDS can be carried out on the basis of a specific immunological assay.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

SUMMARY OF THE INVENTION

However, it has been unexpectedly found that IgA levels were significantly and consistently higher in ALTE or "near miss" SIDS cases. IgA can therefore be used as a predictor of susceptibility to development of ALTE and/or SIDS.

According to a first aspect, the invention provides a method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:

a) determination of IgA level in a sample from a subject; and

b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA level with a predetermined standard.

According to a second aspect, the invention provides a method of assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:

a) determination of IgA1 level in a sample from a subject; and

b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA1 level with a predetermined standard.

According to a third aspect, the invention provides a method for assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:

(a) determination of salivary IgA level; and

(b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA level with a predetermined standard.

According to a fourth aspect, the invention provides a method for assessing potential susceptibility to development of ALTE and/or SIDS in a subject including:

(a) determination of salivary IgA1 level; and

(b) prediction of susceptibility to development of ALTE and/or SIDS by comparison of said IgA1 level with a predetermined standard.

According to a fifth aspect, the invention provides use of the measurement of IgA as a predictor of susceptibility to development of ALTE and/or SIDS.

According to a sixth aspect, the invention provides use of the measurement of IgA1 as a predictor of susceptibility to development of ALTE and/or SIDS.

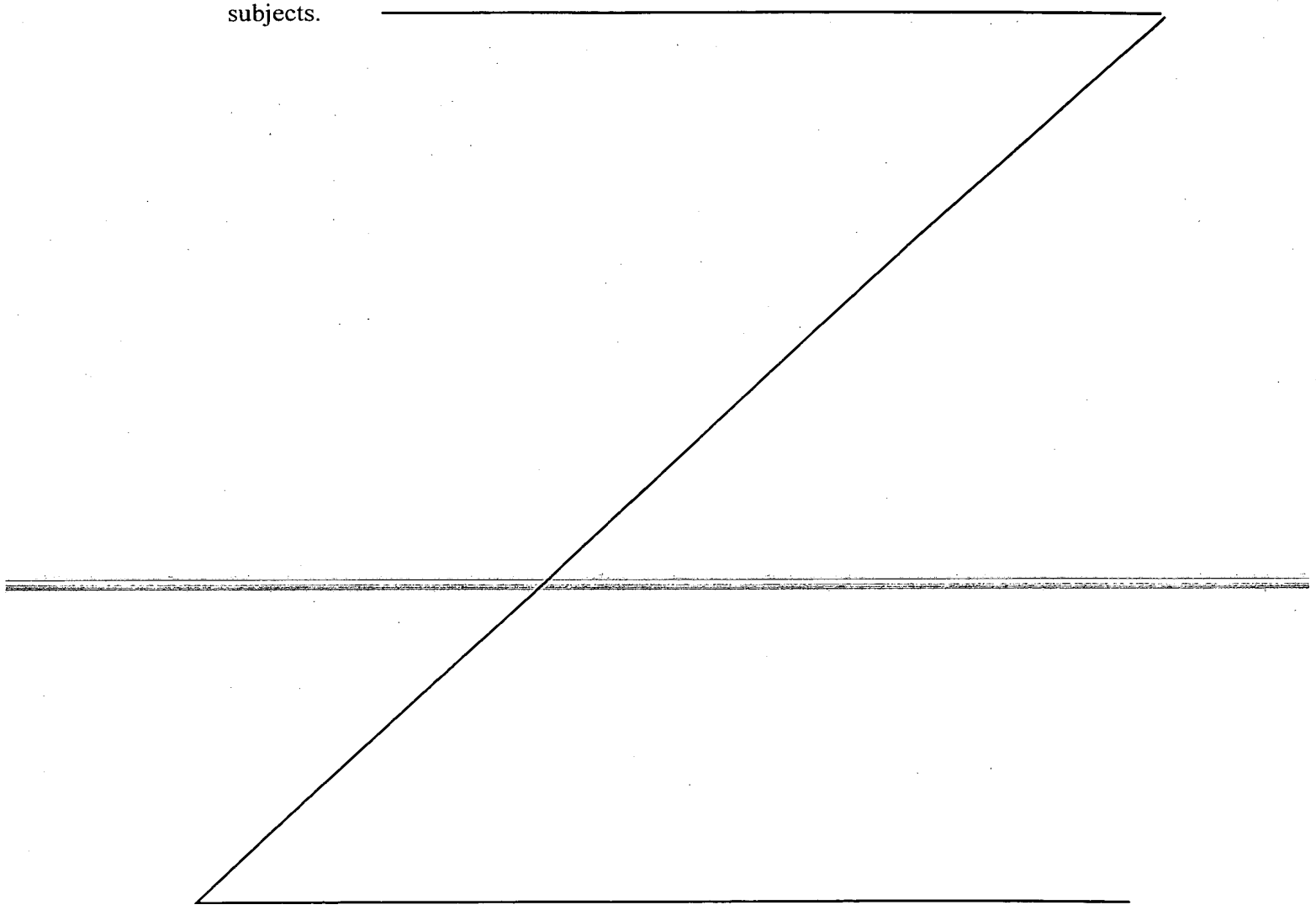
Preferably, the sample is a sample from a subject at the time of, or up to approximately 2 weeks after, an upper respiratory tract infection (URTI). Preferably, the subject is a human infant.

Preferably the sample contains secretory IgA and preferably the sample is saliva. When the sample is saliva, preferably the saliva is whole unstimulated saliva. Preferably the subject is not fasting when the saliva is collected. Other body secretions known to contain IgA would also be useful samples for the present method. The sample need not necessarily be removed from the subject but the method may be applied *in situ*, for example by contacting an assay system on a solid support with a body secretion. It will be understood that any assay system suitable for *in situ* testing would be appropriate.

Preferably, the IgA or IgA1 level is determined by ELISA. However, it will be understood that other suitable methods can be employed such as radial immunodiffusion, RIA and similar methods, all of which would be known to a skilled addressee. The method is particularly suitable for an assay of IgA or IgA1 which is a near subject, rapid yes/no test for immediate action.

It will be clear to the skilled addressee that the measurement of IgA or IgA1 could also be used as a predictor of susceptibility to development of ALTE and/or SIDS in conjunction with other indices such as other immunoglobulins, for example IgM or IgG, acute phase reactants or cellular components.

In the context of the present invention, the word "standard" includes within its meaning, but is not limited to, the average IgA or IgA1 value for age-matched normal subjects.



BRIEF DESCRIPTION OF THE FIGURES

Figure 1 IgA concentration levels (mg/L) for ALTE, "mild" and well infants - initial sample.

Figure 2 IgM concentration levels (mg/L) for ALTE, "mild" and well infants - initial sample.

Figure 3 IgG concentration levels (mg/L) for ALTE, "mild" and well infants - initial sample.

Figure 4 IgA concentration levels (mg/L) for ALTE, "mild" and well infants - 14 day sample.

Figure 5 IgM concentration levels (mg/L) for ALTE, "mild" and well infants - 14 day sample.

Figure 6 IgG concentration levels (mg/L) for ALTE, "mild" and well infants - 14 day sample.

DESCRIPTION OF THE INVENTION

A preferred embodiment of the invention will now be described, by way of example only.

EXAMPLE

Saliva Collection: Whole mixed saliva was collected by gentle suction from the buccal cavity of the mouth [15]. This technique is successful in children (aged from 1 day) and adults [1,16].

Questionnaire: A standardised questionnaire was used to collect the relevant SIDS related demographics. The classification into the "near-miss" SIDS group (ALTE) was made by the attending paediatrician on the basis of clinical investigations.

Saliva Tests: Salivary immunoglobulins were measured by ELISA and albumin by rate nephelometry (Beckman, ARRAY) [16].

Statistical Analysis: The differences in mucosal immune parameters was determined between the ALTE infants and two control groups of subjects (mild URTI and well infants) using analysis of variance (ANOVA) or the appropriate non-parametric statistics.

SUBJECTS

There were 37 subjects aged 1-10 months recruited (20 males, 17 female) in 3 categories:

- Acute life Threatening episodes (ALTE) at John Hunter Hospital (n=5)
- Mild respiratory tract illness (MILD) from General Practitioners (n=11)
- A well control group (WELL) from immunisation clinics (n=21).

QUESTIONNAIRE DATA

- There were more males (n=4) than females (n=1) in the ALTE group.
- There were no significant differences between the groups for age, birth history, family demographics, ethnic background or family history of SIDS.
- There were a higher percentage of children exposed to passive tobacco smoke (60%, n=3) in the ALTE group compared to the MILD (36%, n=4) and WELL (10%, n=2) control groups ($p=0.03$).
- The ALTE group had a higher percentage of families in the average-below average socio-economic category (100%) compared to the other control groups ($p<0.01$).
- There were no differences between the groups for feeding history, immunisation status, sleeping position.

- In 4 of the 5 ALTE subjects an Upper Respiratory Tract Illness (URTI) was suspected as the cause of the ALTE (Table 1).

TABLE 1

Doctor Questionnaire - ALTE

	IgA (mg/L)		Q12	Q32	Q34	Q40	Follow Up
Study Number	Initial	14 Day	Face Covered	Prior URTI	Passive Smoke	URTI Suspected	Suspected Clinical Cause
A01	115.5	56.1	N	N	Y	N	Gastro-esophageal reflux
A02	228.9	22.8	N	Y	Y	Y	RSV+ve Bronchiolitis
A03	410.6	230.9	N	Y	Y	Y	RSV+ve Bronchiolitis
A04	91.0	28.9	Y	Y	N	Y	RSV+ve Bronchiolitis
A05	26.6	79.2	N	Y	N	Y	Reflux with aspiration

SALIVARY IMMUNOGLOBULINS

Two samples of saliva were collected from each subject. The first sample was collected from the ALTE group within 24 hours of admission to hospital and from the MILD respiratory illness group within 48 hours of presentation of their General Practitioners. The WELL group were recruited from immunisation clinics and saliva collected at ages to approximate the ages of presentation of the ALTE and MILD groups. The second sample was collected 14 days later from each subject.

The figures in Appendix C have the age related 5^{th}-95^{th} percentile reference ranges indicated for each salivary immunoglobulin over the first year of life.

- The salivary IgA, IgG and IgM concentration in the ALTE group were all significantly higher than the MILD (Tables 2A and 2B) and WELL (Tables 3A and 3B) groups for both sample 1 and 2 (Figures 1 and 2).
- There were no significant differences between the MILD and WELL groups for either sample 1 or sample 2 (Tables 2C and 3C).
- There were two subjects in the MILD group who had grossly elevated salivary immunoglobulin concentrations in the 14 day collections. (See Appendix C).
    - RO3 had an elevated IgA 12 days post infection. No reason could be identified.
    - RO9 had an elevated IgM that is most likely accounted for by immunisation with Triple antigen and *Haemophilias influenzae* B 14 days prior to the saliva collection.

TABLE 2A
First Sample
Analysis of Immunoglobulins - ALTE vs MILD

	ALTE			MILD			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	115.55	(27-411)	11	9.93	(0-37)	<0.01
IgG	5	9.21	(0-16)	11	0.00	(0-3)	0.02
IgM	5	4.61	(3-24)	11	2.18	(0-16)	0.04

TABLE 2B
First Sample
Analysis of Immunoglobulins - ALTE vs WELL

	ALTE			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	115.55	(27-411)	21	11.37	(0-67)	<0.01
IgG	5	9.21	(0-16)	21	0.00	(0-8)	0.01
IgM	5	4.61	(3-24)	21	1.00	(0-33)	0.01

TABLE 2C
First Sample
Analysis of Immunoglobulins - MILD vs WELL

	MILD			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	11	9.93	(0-37)	<0.01	11.37	(0-67)	0.68
IgG	11	0.00	(0-3)	0.02	0.00	(0-8)	0.66
IgM	11	2.18	(0-16)	0.04	1.00	(0-33)	0.36

TABLE 3A
Second Sample
Analysis of Immunoglobulins - ALTE vs MILD

	ALTE			MILD			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	56.06	(23-231)	11	8.88	(1-255)	0.04
IgG	5	2.99	(2-7)	11	0.00	(0-4)	0.03
IgM	5	9.39	(2-16)	11	2.31	(0-27)	0.07

TABLE 3B
Second Sample
Analysis of Immunoglobulins - ALTE vs WELL

	ALTE			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	5	56.06	(23-231)	20	10.53	(0-58)	<0.01
IgG	5	2.99	(2-7)	20	0.00	(0-6)	<0.01
IgM	5	9.39	(2-16)	20	1.66	(0-14)	<0.01

TABLE 3C
Second Sample
Analysis of Immunoglobulins - ALTE vs WELL

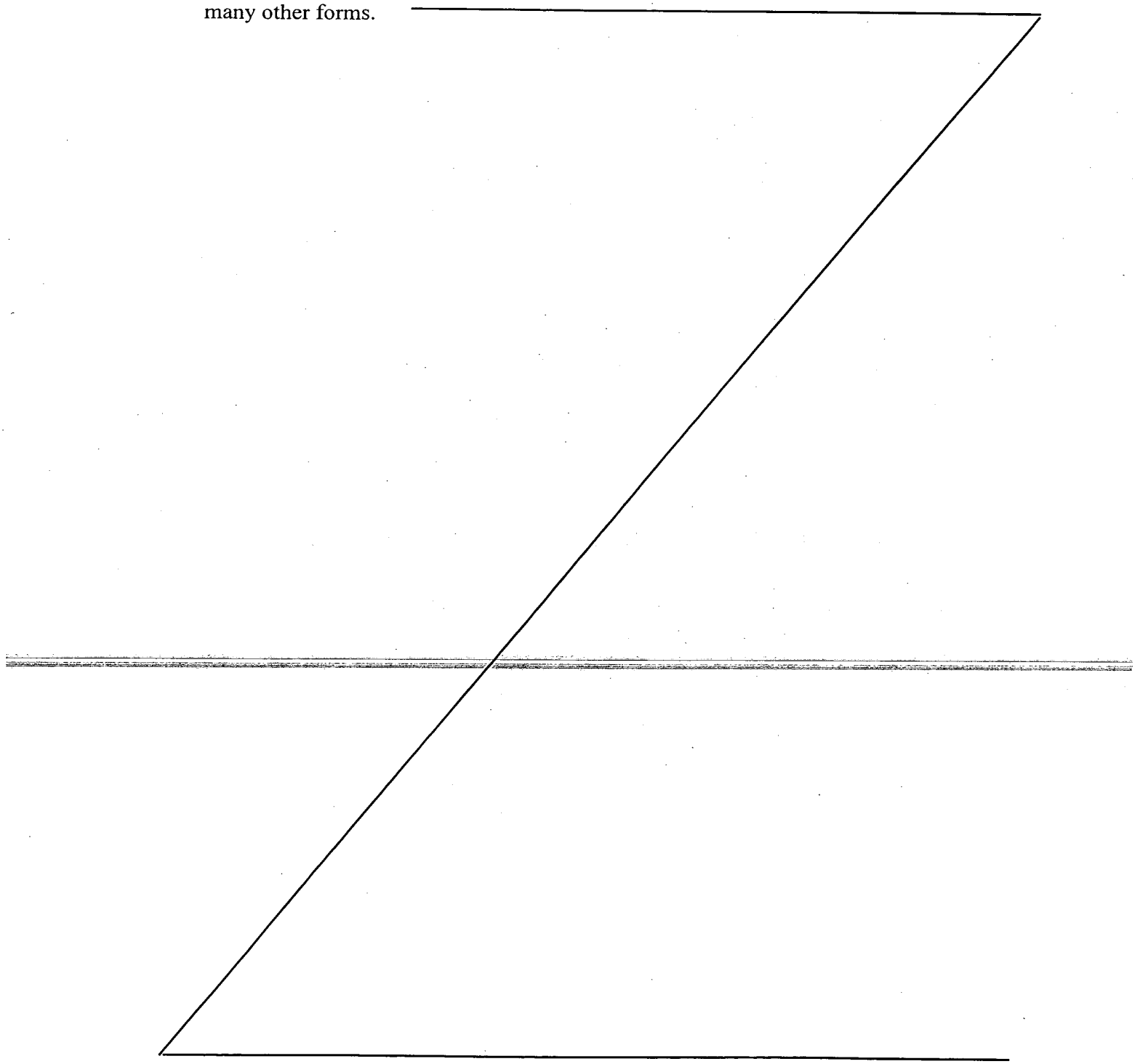
	MILD			WELL			
	N	Median	Range	N	Medial	Range	P-value
IgA	11	8.88	(1-255)	20	10.53	(0-58)	0.71
IgG	11	0.00	(0-4)	20	0.00	(0-6)	0.75
IgM	11	2.31	(0-27)	20	1.66	(0-14)	0.56

CONCLUSIONS

- The grossly elevated salivary IgA concentration in 4 of 5 ALTE subjects at presentation was an unexpected result and was not observed in the MILD or WELL control groups. Salivary IgA can therefore act as a marker for ALTE (and SIDS) in subjects presenting with an otherwise mild respiratory illness.
- The elevated salivary IgA and IgM concentrations in 4 of 5 ALTE support the concept of an infection or inflammatory cause in ALTE (and SIDS).

- RSV positive Bronchiolitis was evident in 3 of 5 ALTE subjects.

Although the invention has been described with reference to specific examples, it will be appreciated by those skilled in the art that the invention may be embodied in many other forms.



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Dated this 7th Day of June, 1999

THE UNIVERSITY OF NEWCASTLE RESEARCH ASSOCIATES (TUNRA) LIMITED

Attorney: IVAN A. RAJKOVIC
Fellow Institute of Patent Attorneys of Australia
of BALDWIN SHELSTON WATERS

Salivary IgA Concentration Levels
for ALTE (□) , Mild (○) and Well (Δ) Infants
Initial Sample.

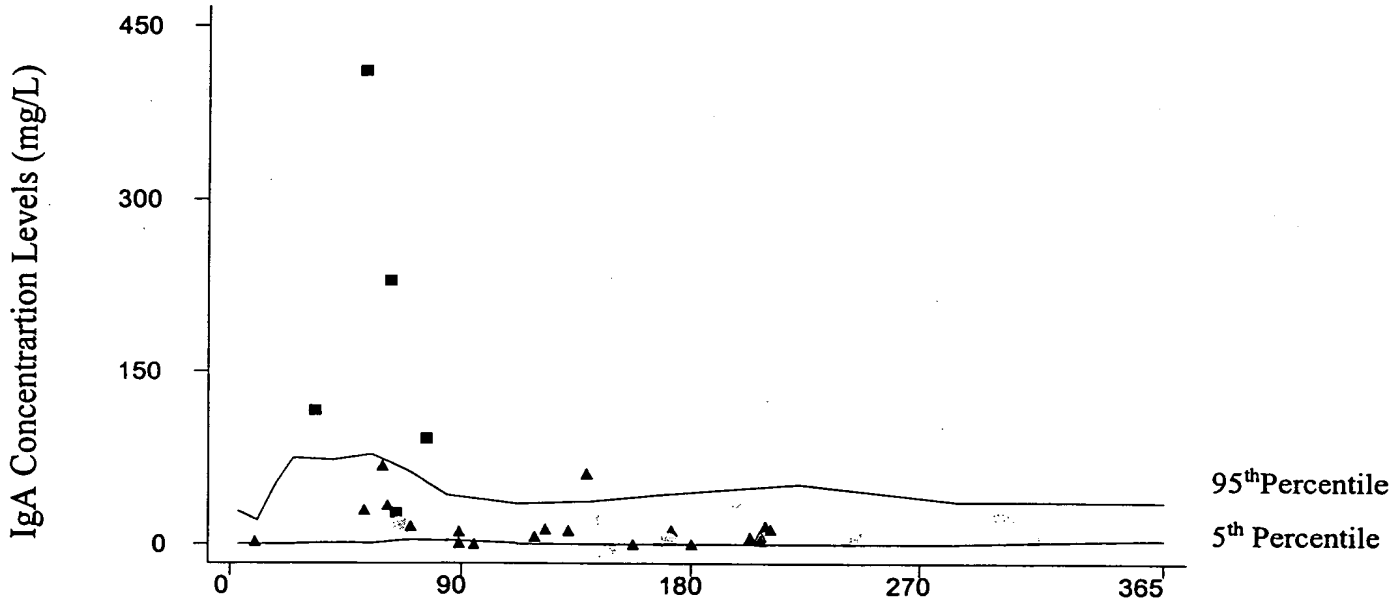
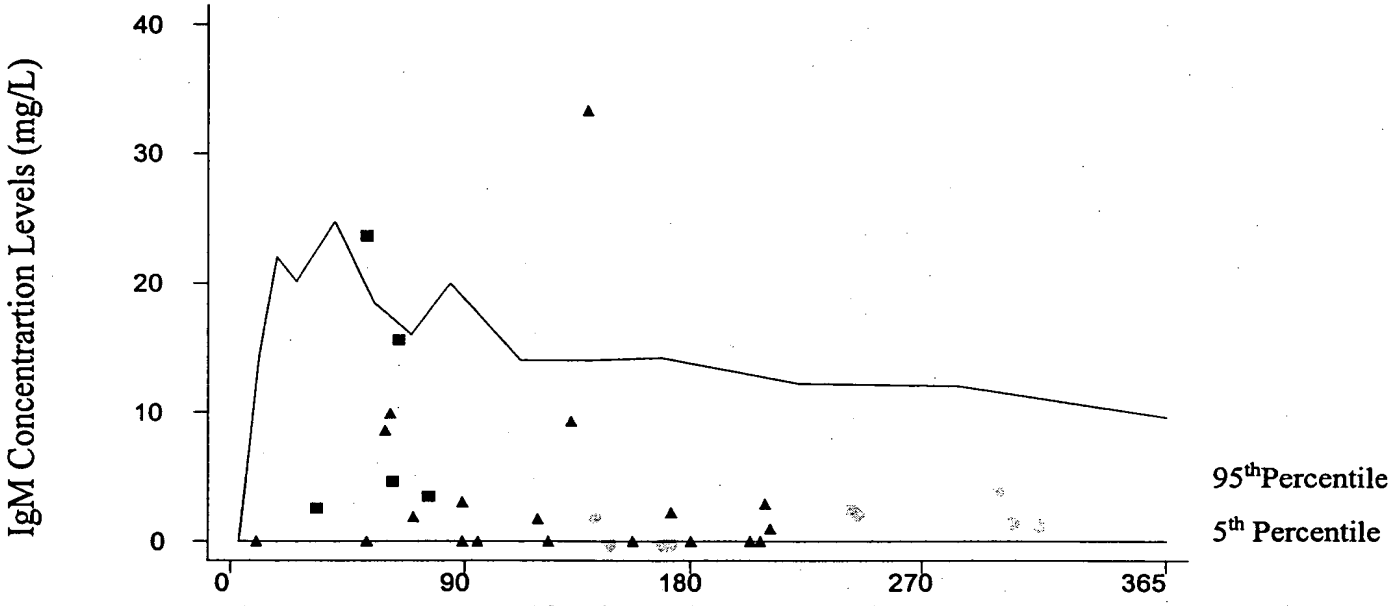


Fig.1

Salivary IgM Concentration Levels
for ALTE (□) , Mild (○) and Well (Δ) Infants
Initial Sample.



Age (Days)

Fig.2

Salivary IgG Concentration Levels
for ALTE (□) , Mild (○) and Well (Δ) Infants
Initial Sample.

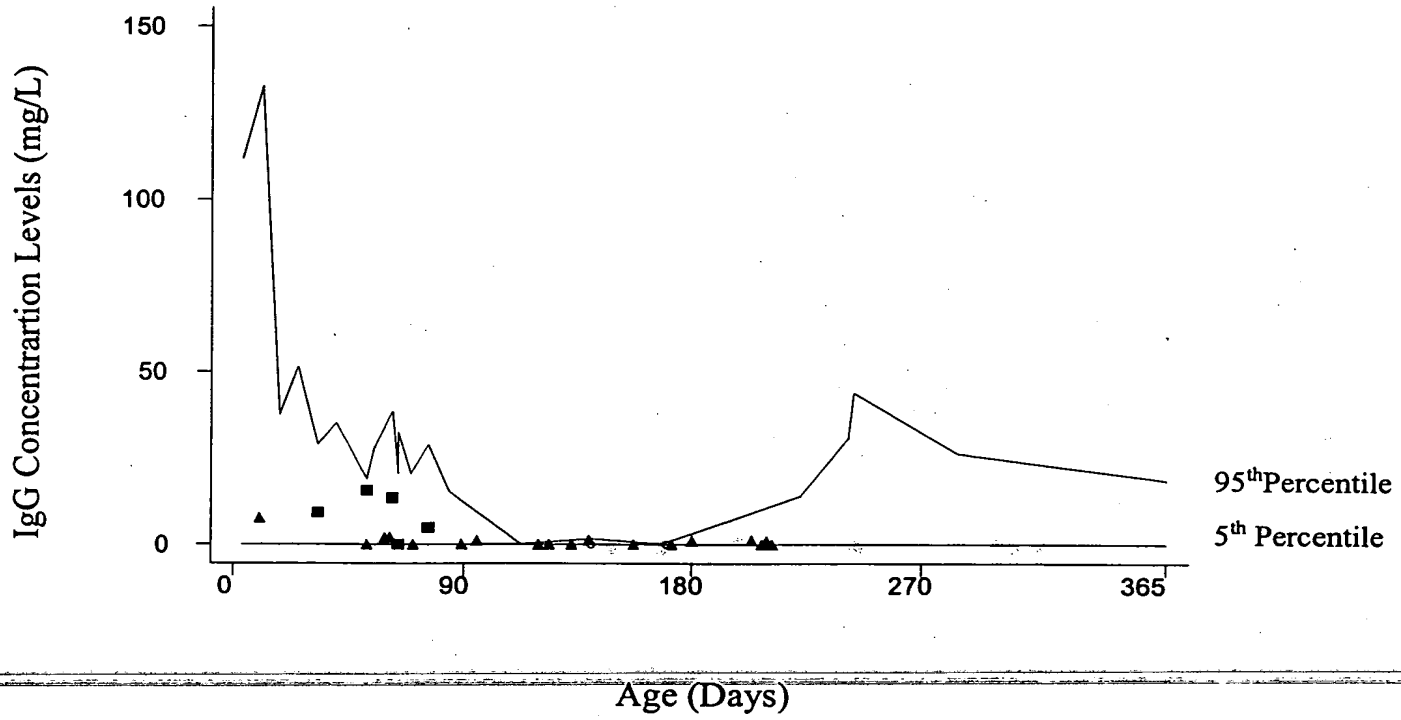


Fig.3

Salivary IgA Concentration Levels
for ALTE (□) , Mild (○) and Well (Δ) Infants
14 Day Sample.

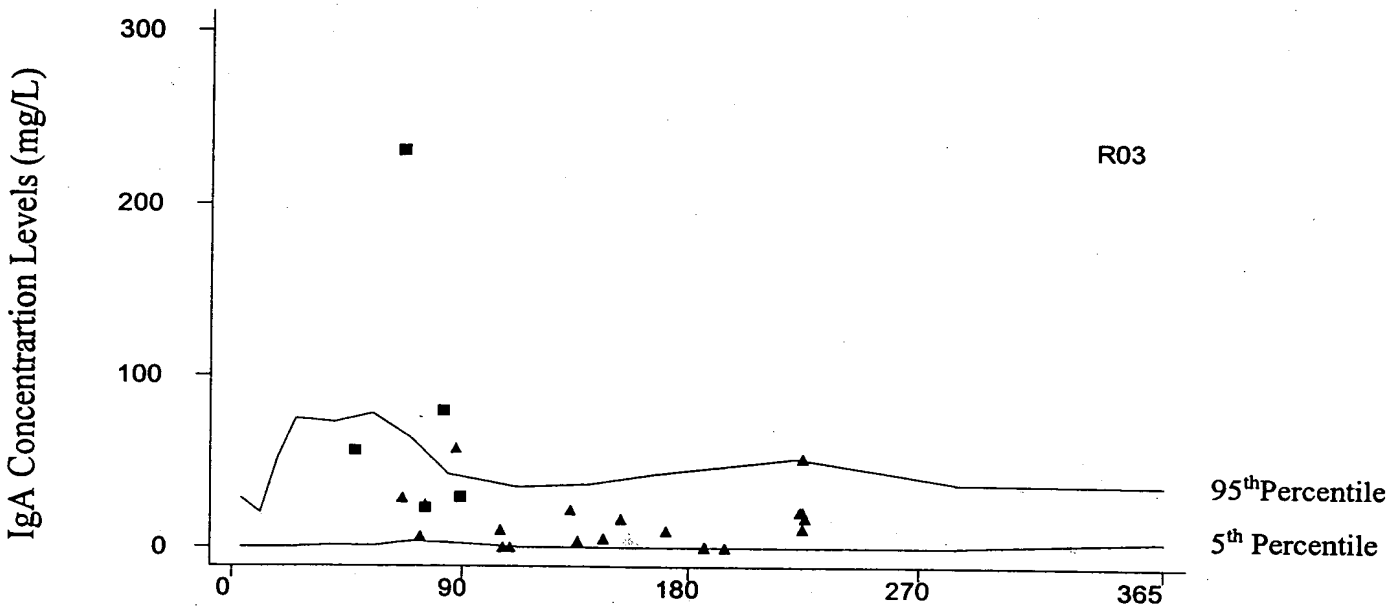


Fig. 4

Salivary IgM Concentration Levels
for ALTE (□) , Mild (○) and Well (Δ) Infants
14 Day Sample.

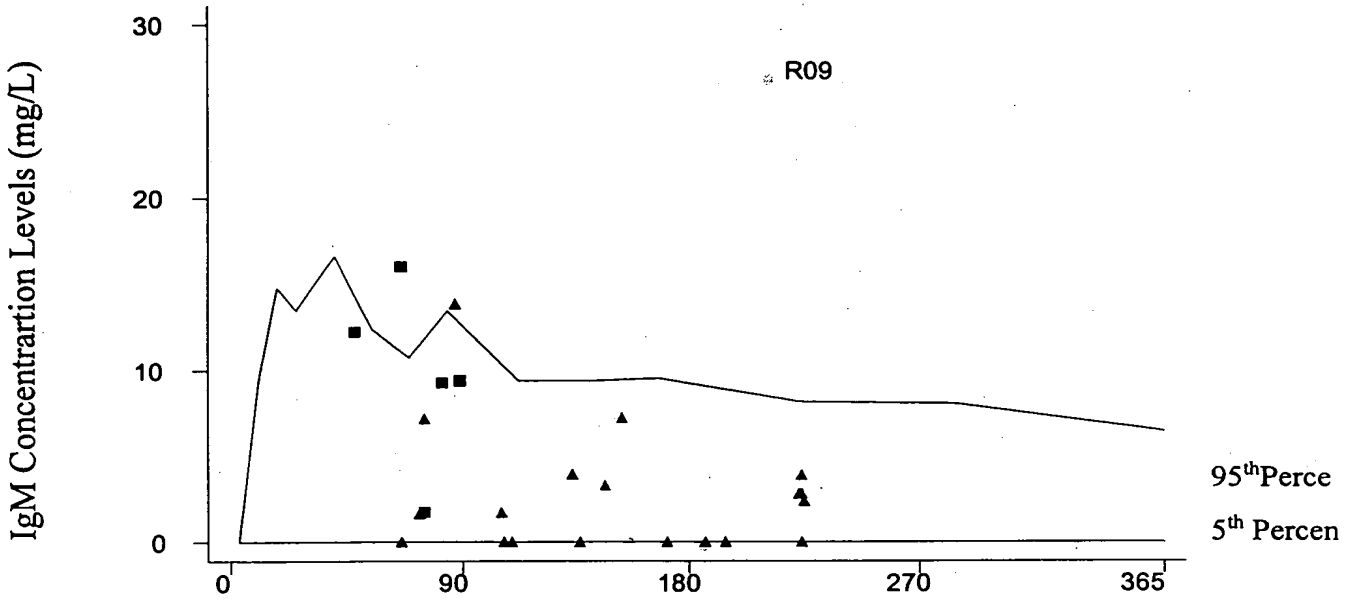


Fig.5

Salivary IgG Concentration Levels
for ALTE (□) , Mild (○) and Well (Δ) Infants
14 Day Sample.

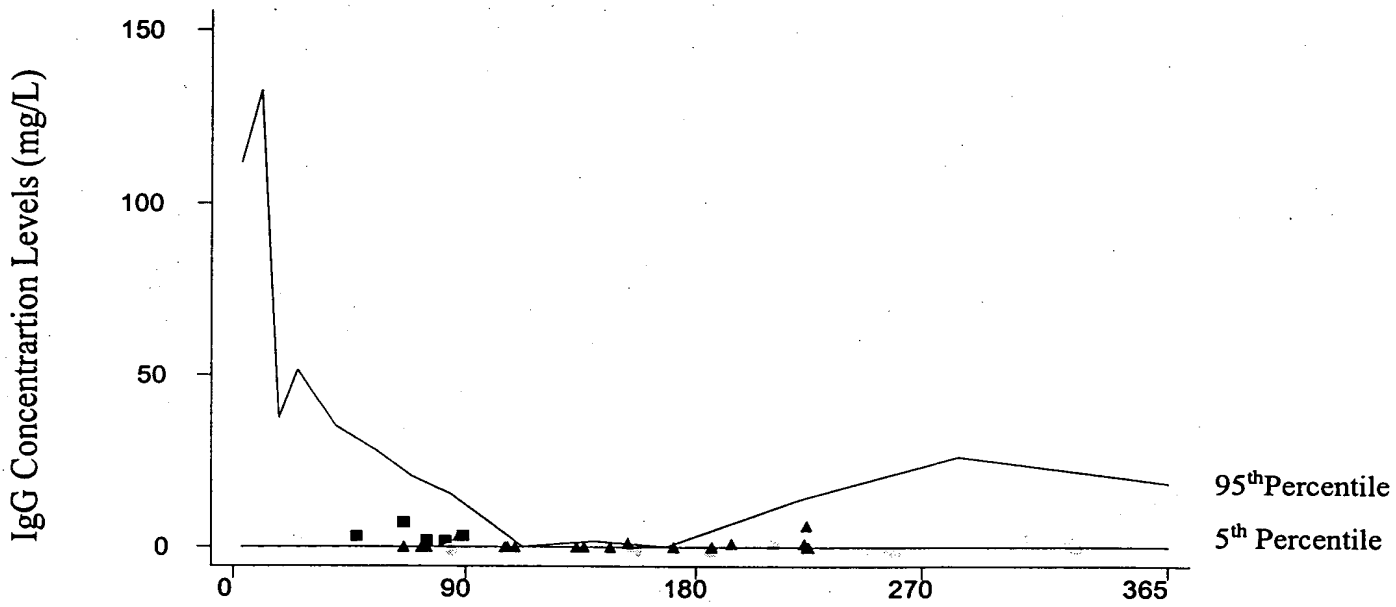


Fig.6